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REVIEW

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Precision-cut intestinal slices: alternative model for drug transport, metabolism, and toxicology research

Ming Li, Inge A. M. de Graaf and Geny M. M. Groothuis

Pharmacokinetics, Toxicology & Targeting, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands

ABSTRACT

Introduction: The absorption, distribution, metabolism, excretion and toxicity (ADME-tox) processes of drugs are of importance and require preclinical investigation intestine in addition to the liver. Various models have been developed for prediction of ADME-tox in the intestine. In this review, precision-cut intestinal slices (PCIS) are discussed and highlighted as model for ADME-tox studies.

Areas covered: This review provides an overview of the applications and an update of the most recent research on PCIS as an *ex vivo* model to study the transport, metabolism and toxicology of drugs and other xenobiotics. The unique features of PCIS and the differences with other models as well as the translational aspects are also discussed.

Expert opinion: PCIS are a simple, fast, and reliable *ex vivo* model for drug ADME-tox research. Therefore, PCIS are expected to become an indispensable link in the *in vitro–ex vivo–in vivo* extrapolation, and a bridge in translation of animal data to the human situation. In the future, this model may be helpful to study the effects of interorgan interactions, intestinal bacteria, excipients and drug formulations on the ADME-tox properties of drugs. The optimization of culture medium and the development of a (cryo)preservation technique require more research.

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Drug–drug interaction; drug metabolism; drug transport; *ex vivo*; precision-cut intestinal slices; toxicology

1. Introduction

The intestine is extensively involved in the transport, metabolism, and toxicity of drugs after oral administration, which requires preclinical investigation. Various *in vivo* and *in vitro* models (shown in Figure 1) have been developed in the past, but each of these applications has several limitations (as discussed in Section 2). In this review, the application of the more recently developed model of precision-cut intestinal slices (PCIS) for drug transport, metabolism, and toxicology research for drugs and other xenobiotics will be discussed.

The model of tissue slices was first invented in 1923 by Otto Warburg to measure cell metabolism and oxygen consumption in tumor tissue [1] and further explored by Hans Krebs to study amino acid metabolism in various organs and species including human.[2] However, the tissue slices at that time had severe limitations, for example, rapid loss of viability and irreproducible thickness, due to the nonoptimized incubation conditions and the fact that they were hand-cut using a razor blade, respectively.

In 1980, Carlos Krumdieck developed a new semiautomatic instrument, the Krumdieck tissue slicer, to reproducibly prepare thin and precision-cut liver slices, [3] and thereby initiated the revival of tissue slices in collaboration with Klaus Brendel and various other members of his laboratory.[4–8] This collaboration resulted in optimization of the tissue viability (e.g. submersion in cold oxygenated buffer during slicing) and increased reproducibility by enabling a well-defined size by the semiautomatic slicing (e.g. 4–15 mm diameter and 100–400 µm thickness). Thereafter, the applications of the precision-cut tissue slices (PCTS) were widely explored not only in the area of cell metabolism in healthy and cancer cells, but also in transport, metabolism, and toxicity of drugs,[9–11] fibrosis,[12–14] obstructive lung diseases,[15–17] and viral infections.[18]

Initially, the precision-cut technique was applied to solid organs such as the liver and the kidney but not applied to the intestine. Due to its structure as a hollow, tender, long, highly differentiated, and delicate organ, it appeared more difficult to slice and incubate. As a result, intestinal slices were initially not produced with a semiautomatic slicing instrument, but with a coring tool and punched directly out of the intestinal wall and thus had the full thickness of the intestinal wall.[5,19]

CONTACT Geny M.M. Groothuis g.m.m.groothuis@rug.nl Pharmacokinetics, Toxicology and Targeting, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Article highlights

- After the introduction of tissue slicer and agarose filling and embedding, precision-cut intestinal slices (PCIS) has been established as *ex vivo* model for the intestine, which can be easily applied to the human intestine and that of various animals.
- As an alternative model, PCIS have an important potential for absorption, distribution, metabolism, excretion, and toxicity studies due to sufficient maintenance of tissue viability, activity and functionality to perform toxicity and induction studies, maintenance of cell polarity and cell–cell and cell–matrix contacts, relatively easy and fast preparation, efficient use of the scarce tissue resulting in 100–200 slices per experiment, applicability to the human situation, and convenience in studying regional and species differences, in compliance with replacement, reduction, and refinement. However, its limitations should also be taken into account.
- PCIS were applied to study drug transport related to efflux transporters (P-glycoprotein (P-gp), multidrug resistance-associated proteins) and influx transporters (apical sodium-dependent bile acid transporter). The *ex vivo* assessment of the transporters involved and the evaluation of the inhibitory potencies of their inhibitors are expected to make more accurate predictions for potential drug–drug interactions (DDIs) *in vivo*.
- Applications of PCIS on drug toxicity were focused on drug-induced gut injury by nonsteroidal anti-inflammatory drugs and toxic bile acids. PCIS are also promising in evaluating the toxicity of anticancer compounds.
- PCIS can be applied to predict DDIs by studying the induction and inhibition of drugs on the activity of drug transporters and drug metabolizing enzymes. Furthermore, the transport–metabolism interplay, for example, P-gp/CYP3A interplay, and inter-organ interactions can be studied in PCIS.

This box summarizes key points in the article.

However, they appeared to lose their viability rather rapidly.[20] Up to now, applications of intestinal punches are limited to studies on the metabolism of cyclosporine A and tegaserod in human intestinal slices [5,6,21] and the metabolic activation of carcinogens in rat colon slices.[22]

The preparation of PCIS was introduced later by de Kanter et al., who used low-gelling agarose to fill the rat intestinal lumen and sliced the intestinal segments perpendicularly to the length of the intestine, resulting in rings of tissue for rat PCIS.[20] This improvement proved to be very valuable as it resulted in a better reproducibility and tissue viability,[23] while at the same time it was relatively simple and convenient to prepare and use.[24] This improved preparation technique for PCIS has been successfully applied to studies on drug metabolism, induction, and inhibition, as reviewed.[25] Later, this technique was applied to the intestine of mouse,[10] human,[26,27] and, most recently, chicken.[28] It should be noted that the preparation is slightly different for human PCIS. Since the muscle layer is too thick and the circumference of the lumen is too big to slice a whole ring of the intestine, slices are produced from sheets of intestinal tissue after the removal of the muscle layer. The detailed procedures of preparation and incubation of PCIS were extensively described by de Graaf et al. [29] and are shown in Figure 2.

2. PCIS as alternative model to the conventional methods

Many methods have been developed and successfully applied to characterize and predict the absorption, distribution, metabolism, excretion, and toxicity (ADME-tox) properties of new chemical entities (NCEs) and xenobiotics (Figure 1).[30–35] *In vitro* cell cultures and *in vivo* animal models are the conventional methods that are widely used in both academia and industry, and they are well characterized. Among the various cell culture models, Caco-2 monolayer culture has been considered as the 'gold standard' in studying intestinal disposition of drugs *in vitro*.[36] However, these cell cultures have some limitations that limit the accuracy of the prediction for *in vivo*. In general, *in vitro* cell cultures do not reflect the tissue multicellularity and three-dimensional (3D) structure and some cell types lose their polarization. Moreover, the expression levels of drug transporters (DTs) and metabolizing enzymes (DMEs) deviate from the natural expression.[37,38] On the other side of the spectrum, the *in vivo* models retain the proper physiological conditions, but the screening capacity is too low

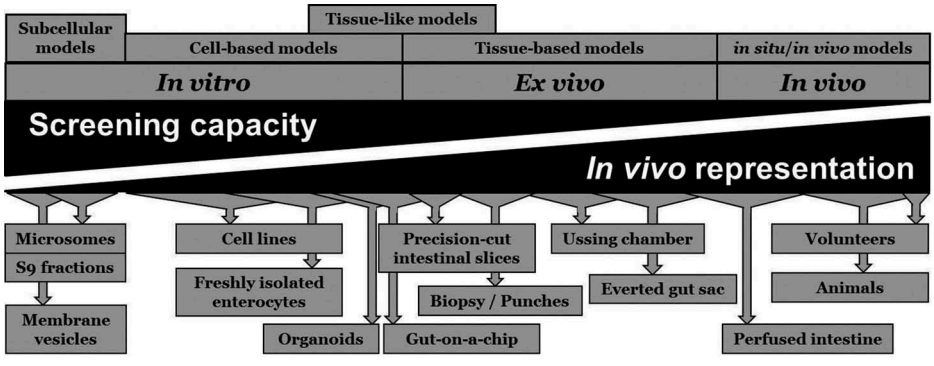

Figure 1. Schematic classification of the current models to study the absorption, distribution, metabolism, excretion, and toxicity properties drugs and xenobiotics in the intestine.

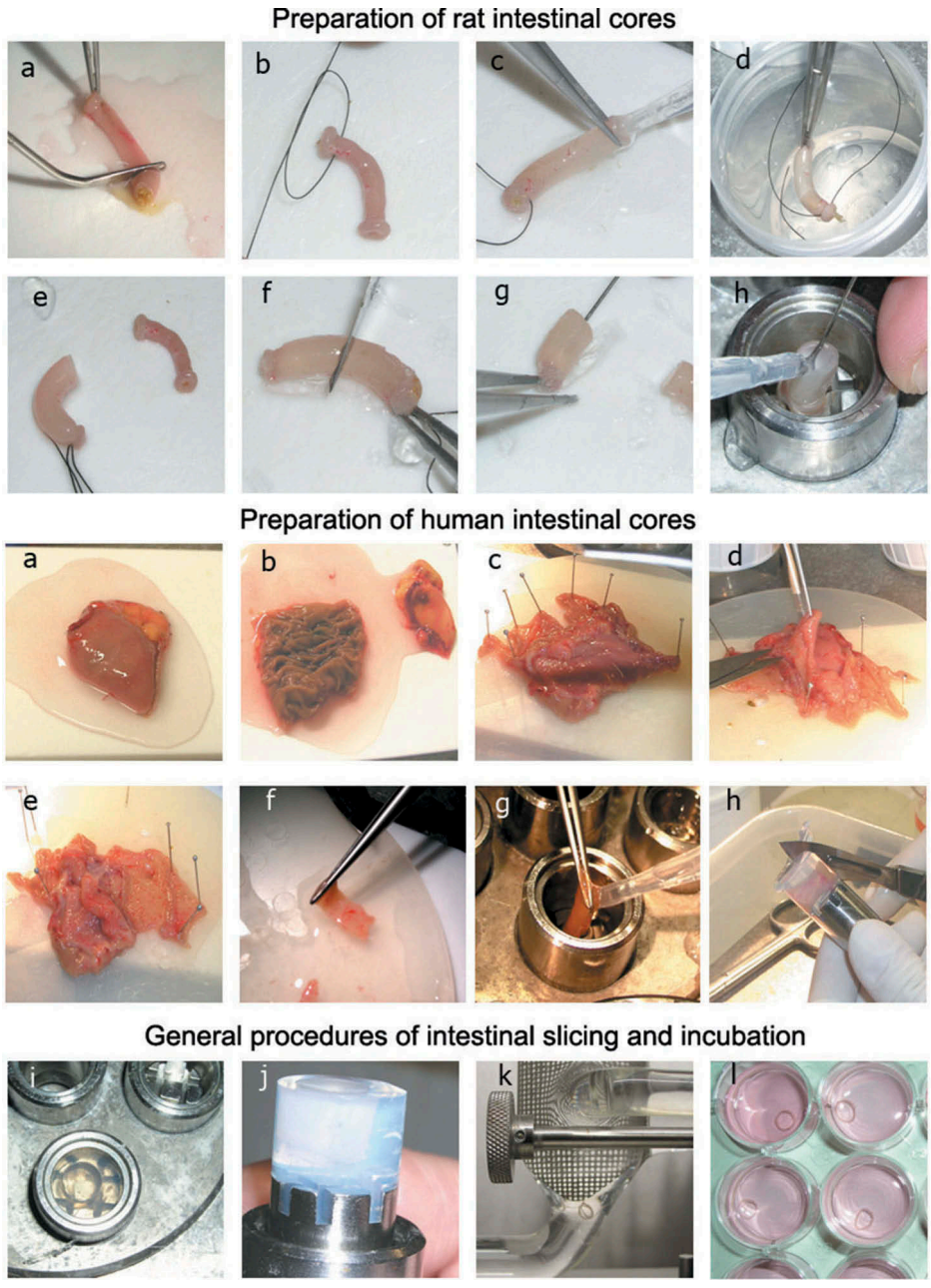

Figure 2. Preparation and incubation of rat and human intestinal slices. Upper panel (a–h): preparation of rat intestinal cores. After the fecal contents are removed from the intestinal segment (a), one side is tied (b). The segment is filled with liquid agarose solution at 37°C (c) and cooled (d) to form a filled cylinder about 5 mm thick (e). After cutting the segments into two halves (f), a pin is placed in the filled lumen (g) to fix the segment in the precooled cylindrical mold plunger (h). The mold is then filled with agarose solution at 37°C. Middle panel (a–h): preparation of human intestinal cores. (a) Piece of human jejunum. Fat tissue is removed and the intestine is opened (here the mucosal side is facing upward) (b). The segment is then fixed on a silicone mattress on the precooled tissue-embedding unit with pins (c) and the muscular is gently cut (stripped) away (d, e). Thereafter, the stripped intestine is cut into pieces of approximately 10 × 20 mm (f) and embedded with low-gelling agarose (g, h). Lower panel (i–l): slicing and incubation of rat precision-cut intestinal slices. Agarose cooled in the mold (i). The plunger is removed from the mold and transferred to the Krumdieck tissue slicer (j). Slices of 2–4 mg in wet weight are cut (k) and incubated in 12-well plates (l). Reproduced with permission from [29].

and the cost, both in animal lives and in money, is much higher. Moreover, applications of these *in vivo* models in human are extremely difficult due to ethical constraints and exceptionally high costs. To fill this gap, alternative methods using intact tissue, such as everted sac, perfused intestinal loops, Ussing chamber, intestinal punches, and, more recently, PCIS, have been developed and are increasingly used to provide additional

information on the intestinal handling of NCEs during preclinical investigation. Recently, the use of stem cells differentiated during culture to organoids has been added to this spectrum of techniques.[39] However, up to now these stem-cell-derived intestinal organoid have not been fully characterized for drug metabolism and transport function, and, moreover, similar to the Caco-2 model, they can at best represent only one specific location in the intestine. Of these models, the PCIS model combines the maintenance of tissue 3D structure and multicellularity and physiological polarized expression of DTs and DMEs with a good level of reproducibility and viability and highly efficient use of scarce tissue and can represent all regions of the intestine. Analogous to the model of precision-cut liver, lung, and kidney slices, PCIS are believed to be a physiologically relevant *ex vivo* model for the intestine, providing valuable information on the ADME-tox functions of the intestine and have recently also been presented as a disease model in fibrosis research.[40]

2.1. Compliance with 3Rs

The principle of 3Rs (replacement, reduction, and refinement) initiated by William Russell and Rex Burch in 1959 [41] encourages the use of alternatives to animal testing in order to reduce the use of experimental animals and also to improve their welfare and the scientific quality of the experiments when the use of animals cannot be avoided. Nowadays, the 3Rs are respected more and more by research scientists and in many countries they are explicitly taken up in the legislation concerning animal use. PCIS seem to be in good compliance with the 3Rs, most notably with the reduction. The number of animals needed can be considerably reduced when using PCIS, as from each region of the intestine within one experiment one can easily make >100 slices, that is, perform >100 tests. In addition, the other organs from the same animal, for example, liver and kidney, can be simultaneously sliced for studies on these organs. Replacement can be achieved by the application of human PCIS, prepared from medical waste tissue derived from surgical resections of patients' intestine. In addition, the use of human PCIS provides direct human data overcoming problematic translation due to species differences. Refinement can be achieved in toxicity studies as the animals do not have to be exposed *in vivo* to the test chemical, but the PCIS are exposed *ex vivo*. The discomfort of the animals is reduced to a minimum as there is only stress due to handing and induction of anesthesia. PCIS can also be made from diseased tissue to study disease mechanisms or potential drugs. It should be noted that in these cases suffering of the animals cannot be avoided completely because it requires animals with induced intestinal disease, for example, dextran sulfate sodium-induced colitis in mice [42] and trinitrobenzene sulfonic acid-induced colitis in rats.[43] However, due to the possibility to perform many studies in one animal, also in these cases the total number of animals with discomfort is reduced.

2.2. Technical considerations

One important feature for a good model is its robustness and reproducibility. As mentioned above, >100 slices can be produced from each region of the rat intestine. Thus, it is possible to perform many series of tests with the slices from all intestinal regions of the same rat and in triplicate, which improves the quality of the results. In addition, the procedures of preparation and incubation of PCIS are relatively simple and do not require very expensive instruments. Based on our own experience, after a short period of training, one researcher can handle a middle-sized experiment (ca. 100 slices) in a few hours, while two researchers are needed for a large experiment (up to 400 slices). Compared to other tissue preparations and *in vivo* animal studies that consume a considerable amount of time, technical and animal handing competences and efforts, PCIS are relatively easy to handle. Moreover, the procedures and protocols for PCIS are standardized and published in *Nature protocols*.[29] For small animals, the procedures are similar to those for rat PCIS, where pieces of intestine are filled with and embedded in agarose before slicing. For larger animals, for example, pig, dog, and monkey, the procedure as described for human PCIS, removing of the muscle layer and embedding sheets of tissue in agarose, should be followed. Slices should have a thickness of maximally 400 µm to enable sufficient substrate and oxygen supply. As a result, it is possible to compare the results from different species and organs,[10,23,25,44–46] both qualitatively and, when the correct scaling factors are applied, also quantitatively.[10,47]

Two incubation systems, the shaken multiwell-plate and the perfused biochip,[48] currently coexist, but no significant differences in ATP levels and metabolic rates were found between the biochip and well plates up to 24 h (unpublished observation).

2.3. Viability of PCIS during culture

For models using tissue preparations, the viability during culture is a crucial issue, which is less of an issue in experiments with cell lines. Tissue viability in the

Ussing chamber is generally limited to 2–4 h [47] and in the gut sac model is <3 h,[49,50] possibly due to a lack of oxygen supply and nutrient penetration into the center of the relatively thick intestinal wall. In contrast, in PCIS from rat small intestine and colon the intracellular ATP content, a general viability marker, is retained until 8 and 24 h of incubation, respectively.[51] In addition, it was found that the slice content of ATP in PCIS of human and mouse small intestine remains at a higher level during 24 h of incubation than in rat PCIS.[27] Consistent with the ATP content, also the morphology of the enterocytes and the epithelium lining remains intact and the mucus production by the goblet cells is maintained, [27,52] although flattening and loss of villi, as well as swelling and edema in the stroma, was observed. Furthermore, live/dead staining (calcein acetoxymethyl ester (calcein-AM)/ethidium bromide) showed that chicken embryo PCIS can remain viable for up to 4 days.[28] Our own observations using fixable-dye viability staining also showed that only a few dead cells could be observed in rat PCIS after 5 h of incubation (unpublished observation). However, although the expression of the housekeeping gene GAPDH remains constant for up to 24 h,[51] the villin expression, generally considered as a marker for enterocytes, decreased during 8–24 h incubation in rat small intestinal slices, in line with the observation of loss of cells and appearance of debris, but was constant in colon slices up to 24 h.[51] Furthermore, data on lactate dehydrogenase leakage and alkaline phosphatase activity of the rat intestinal slices also supported that viability can be retained in rat PCIS for at least 8 h. [27,51,52] The viability of rat PCIS during incubation is also dependent on the preservation method applied between the removal from the body and the preparation of the cores. Both the method of preparation of the tissue and the composition of the preservation solution appeared to influence the viability of slices after 6 h of preservation. Enriched culture medium (William's medium E supplemented with additional buffering, colloids, and impermeants) appeared to protect the tissue better than the organ preservation solution University of Wisconsin.[53]

In general, rat PCIS also maintain a better viability than rat punched intestinal slices, based on ATP, RNA quality and morphology, no matter if the muscle layer in the intestinal punches was stripped off or not.[20] It can be speculated that the thickness of the punches of intestinal tissue was too large to allow efficient supply of oxygen and substrates to all the cells. Nevertheless, the biotransformation activities of both in phase I, for example, 7-ethoxycoumarin (7-EC) metabolism by cytochrome P450 (CYP)1A, and phase II, for example, 7-hydroxycoumarin (7-HC) by UDP glucuronosyltransferase (UGT) and sulfotransferase (SULT), were comparable in the first 3 h of incubation.[20]

2.4. Functionality of PCIS during incubation

In general, good activities of DMEs and recently also DTs in human and rat PCIS have been found. As mentioned above, the PCIS were shown to have similar phase I and II metabolic activities as fresh punches,[20] but substantially higher (ranging from 3-to 30-fold) than microsomes or S9 fractions prepared from the intestine, after scaling based on the relative protein content.[10] Both phase I and II metabolism of several substrates were shown to be linear during 180 min of incubation,[52] whereas the metabolic rates remained constant up to at least 8 h.[51] The stability of metabolic activity up to 8 h was in line with the reported well-preserved viability and indicated the unchanged abundance of the proteins involved in drug metabolism. After 24 h of incubation, most phase I and II reactions decreased significantly in rat and human jejunum PCIS, while phase II conjugation was well preserved in the PCIS from colon.[27,51]

The first data on the activity of DTs in PCIS became available in 2011,[54,55] but no study has been published yet on the stability of transport activity during culture. Isolation of RNA for RT-PCR after 48 h of incubation results in highly intact RNA,[40] and the expression of several genes coding for DMEs and DTs after 24 h of incubation was shown to be regulated by well-known ligands of transcription factors, indicating fully active gene transcription.[27,56–58] The mRNA expression of some genes like multidrug resistance-associated protein (MRP)2 [59] and apical sodium-dependent bile acid transporter (ASBT) [56] decreases during 24 h of incubation, whereas others like MRP3 [59] increase. Probably, this is a result of lack of appropriate ligands for transcription factors in the medium. On the other hand, it was also noticed that in the slices, even after short incubation periods, a pro-inflammatory process was induced, indicated by increased expression of genes such as TNFα and IL6.[60] Possibly, this was caused by the cold ischemia of the slices during preparation and subsequent reperfusion during culturing. In addition, Pham et al. recently reported on the upregulation of genes associated with the initiation of fibrosis after 24 h of culture.[40] This indicates that PCIS may in the future be used as disease model to test efficacy of antifibrotic drugs.

2.5. Regional differences

The intestine is a heterogeneous organ in which the regional differences in structure and function, including the expression of DTs and DMEs, are prominent.[61,62] Most of these differences have been found by analyzing mRNA expression or protein abundance. However, it is largely unknown to what extent the differences in expression levels (mRNA and/or protein abundance) of DTs and DMEs also represent their functional differences. A discrepancy between protein abundancy and transport activity can be anticipated as it has been demonstrated that the transporters are not solely localized at the plasma membrane but also intracellularly.[63] PCIS offer an excellent opportunity to study these regional differences at the level of activity of DTs and DMEs and to predict the consequences of the heterogeneity of activity and local drug concentration in the lumen on the intracellular and systemic exposure to drugs and their metabolites. It is evident that neither cell lines nor stem-cell-derived organoids can represent these regional differences.

When using tissue of experimental animals, all regions can be studied with tissue from one animal in one experiment with PCIS. However, the likelihood of obtaining tissue from several regions of the human intestine is not high. Most tissue is obtained from patients undergoing partial resections, and the tissue remaining for research after sampling for the pathologist is usually limited to one of the regions. Therefore, due to the large interindividual differences in humans, one needs at least tissue from five or more patients for each region before a significant conclusion can be drawn on regional differences in the human intestine.

2.6. Species differences

Isoforms, expression levels and activities of DTs and DMEs, are considerably different between animals and humans.[46] For this reason, unexpected issues related to ADME-tox of new drugs form one of the major hurdles during preclinical investigations. Methods using human tissue could overcome extrapolation difficulties between humans and animals, but many *ex vivo*/*in vivo* models are impossible to perform with humans for ethical reasons or are technically difficult and expensive.[64] The PCIS method can be easily applied to human tissue since practically the same technique is used for all species (see Section 2.2). Data on transport, metabolism, and toxicity in human PCIS are now slowly appearing in the literature (see below), and more quantitative differences between animal and human data will be identified in the near future. Furthermore, how these results correlate with *in vivo* data and how they can be applied in the drug development process should be further explored.

3. Application of PCIS on drug transport, metabolism, and toxicity

Although the awareness of the importance of the intestine in drug ADME-tox has been rising in the last decade,[65–67] PCIS are still used less often than slices of liver and kidney. In the past decade, PCIS were developed as *ex vivo* model to study drug metabolism and toxicity [10,11,20,27,51,52,68] and, most recently, drug transport.[54,55]

3.1. Application of PCIS for drug transport

Recently, studies were initiated to measure DT activity in PCIS, but they are still very limited to date. Many influx transporters are expressed on the apical membrane of intestinal epithelial cell and have important physiological functions. The activity of uptake transporters can be measured directly by analysis of the increase in slice content after incubation with different concentrations of the substrate and after different time points. Active and passive uptake can be distinguished by incubation at 4°C and 37°C and specific inhibitors can be used to identify the transporter involved. Recently, the uptake of bile acids in PCIS was studied using cholic acid, deoxycholic acid (DCA), and taurocholic acid.[69] In PCIS of rat and human ileum, the active uptake mediated by ASBT could be clearly distinguished from passive uptake by parallel incubation at 37°C and 4°C, and as expected, the involvement of active uptake was larger for the hydrophilic taurocholic acid than for the other two more hydrophobic bile acids. The uptake in the PCIS of jejunum and colon appeared mostly mediated by passive diffusion, which is in line with the fact that ASBT is specifically expressed in the ileum and virtually absent in the other intestinal regions. These data indicate the applicability of PCIS for the study of the activity of uptake transporters.

Direct measurement of excretion from slices into the medium is hampered by the relatively large volume of medium compared to the low amount of tissue and is thus limited by the detection method for the substrate. Therefore, up to now the involvement of efflux transporters has only been studied indirectly by the accumulation of the substrate in the PCIS and the influence of inhibitors of the transporter on the accumulation. Possidente et al. used this method to show the applicability of rat jejunum PCIS for the interactions of xenobiotics with efflux transporters, using calcein-AM as

probe, which is a substrate of P-glycoprotein (P-gp) and is metabolized in the cells to calcein, which is a substrate of MRPs.[55] Inhibitors of both P-gp and MRPs exhibited their inhibitory effects by increasing the intracellular retention of calcein. The enhanced accumulation was concentration dependent and the potency of inhibitors could be quantified as apparent half maximal inhibitory concentration (IC_{50}) values.

P-gp activity along the different regions of the intestine and the inhibitory potencies of several drugs was studied in rat [54] and human (manuscript in preparation) PCIS *ex vivo* using rhodamine 123 (R123), a specific P-gp substrate. Using fluorescence microscopy, it could be confirmed that the enterocytes in the PCIS are the cells involved in the transport of the P-gp substrate. Regional differences in P-gp activity were demonstrated in rat and human PCIS by the increase in accumulation in the tissue in the presence of an inhibitor. The P-gp activity was found to be in ileum > jejunum > duodenum ≥ colon. The increase in tissue accumulation correlated with the level of P-gp expression shown *in vivo*.[70] Interestingly, the regional differences of P-gp activity were larger in the human intestine than in the rat tissue. This shows the relevance of the use of human tissue for more accurate predictions from *in vitro* to *in vivo*. The rank order of the inhibitory potency of several well-known Pgp inhibitors, reflected by the IC_{50}, was consistent with literature reports and comparable in rat and human PCIS, suggesting relatively little differences in the affinity of these inhibitors for human and rat P-gp. This is in line with the findings of Sugimoto et al., who found similar inhibition of rat and human P-gp by verapamil, ketoconazole, and quinidine, but not for cyclosporine A. [71] The IC_{50} obtained in PCIS studies is an apparent IC_{50} as it does not reflect the concentration at the active site of the transporter, but takes into account the uptake, metabolism, and excretion of the inhibitor. This makes the IC_{50} obtained in PCIS a more physiologically relevant parameter as these processes also play a role *in vivo*.

In a similar study, the P-gp-mediated excretion of quinidine, a selective P-gp substrate as well as CYP3A4 substrate, was studied in rat (manuscript in preparation) and human [72] PCIS. Similar to R123, the quinidine accumulation could be enhanced by specific and nonspecific P-gp inhibitors. Although quinidine undergoes metabolism by CYP3A4, the intracellular content of quinidine appeared to be mainly determined by transport by passive diffusion and active P-gp efflux. Interestingly, P-gp inhibition resulted in a marked, more than expected based on the increased quinidine concentration, increase of the intracellular quinidine hydroxyl metabolite concentration, indicating that this metabolite apparently is also a P-gp substrate.

These results show that PCIS are a promising tool for further studies to elucidate the involvement of DTs for new drugs along the length of the intestine. However, it should be noted that no distinction can be made between transport by basolateral and apical transporters for a compound that is a substrate of both, unless specific inhibitors are used. As it was evident from the studies with quinidine, the accumulation of the probe in PCIS can be influenced by other related process, like other DTs and DMEs. Thus, the use of selective inhibitors is critical for identification of the transporters involved. However, this complexity of the physiological conditions also indicates the limited relevance of Caco-2 and other cell lines for the prediction of *in vivo* transport. To date, no comparison between transporter activity *ex vivo* and *in vivo* is available yet.

3.2. Application of PCIS for drug metabolism

Drug metabolism in the intestine has been known for decades but was underestimated for a long period of time. However, studies with human PCIS have shown that the activity of CYP3A4 calculated per enterocyte of the human small intestine may even be similar to or higher than in the hepatocytes. In general, it became evident that intestinal metabolism plays a more prominent role relative to the liver in man than in the rat.[47] Also, Martignoni et al. [10] reported in studies with rat and mouse precision-cut liver and intestinal slices that the metabolite formation rate (expressed per mg protein) of most CYP3A substrates was higher in liver slices than in PCIS, but the formation of 3OH-quinidine was threefold higher in rat intestinal than in rat liver slices, while that of nor-verapamil was comparable. Taking into account that the enterocytes are exposed to much higher concentrations of drugs and xenobiotics taken orally than hepatocytes, their contribution to first-pass metabolism can be very high and is thus relevant to study.

Van de Kerkhof et al. [52] were the first to investigate gradients of drug metabolism along the intestinal tract with PCIS using three model compounds, covering several phase I and II metabolic routes, that is, 7-EC (substrate for CYP1A mainly), 7-HC (substrate of UGT and SULF), and testosterone (substrate for several CYP-isoforms and hydroxysteroid dehydrogenase). As reviewed by van de Kerhof et al.,[47] gradients in phase I and II metabolism from the duodenum toward the colon were shown to be clearly different for the different enzymes: CYP3A4, 2C8, 2C9, and CYP2D6 are highest in the proximal regions of the small intestine and decrease toward the colon, whereas CYP2S1 is

equally expressed in all regions. In contrast, CYP2J2 and CYP3A5 as well as many (but not all) of the UGT and SULT isoforms show an increasing gradient with higher activity in the distal part.

The biotransformation of diclofenac into the 4′-OH and 5-OH metabolites and acyl glucuronide diclofenac was investigated in rat [73,74] and human PCIS [75] and also showed a higher rate of metabolism in human than in rat tissue. Moreover, the different metabolic routes could be specifically inhibited by specific inhibitors. In contrast, the hydroxylation of a cannabinoid receptor 1 antagonist was higher in rat than human intestine.[76] In the latter study, the effect of drug formulation on the metabolite formation was evaluated as a surrogate end point for absorption. The enhanced dissolution rate of nanoparticles resulted in a higher uptake rate and, consequently, in an increased metabolite formation, which was in line with the results from Ussing chamber using human tissue and rats *in vivo*, which indicates a potential new application of the use of PCIS for absorption studies.

3.3. Application of PCIS for the study of drug–drug interactions

Drug–drug interactions (DDIs), as a result of the induction or inhibition of DTs and DMEs as well as due to transport–metabolism interplay, can considerably influence the ADME process.[77] Thus, as the effect of DDIs depends on both the relative affinity of the different drugs and the abundance of the proteins involved, the presence and physiological abundance of all DTs and DMEs, and the required cofactors, is critical for the accurate evaluation of the risk of potential DDIs. Many exogenous and endogenous compounds, such as the ligands of nuclear receptors (NRs), can regulate the expression of DTs and DMEs in the intestine. Several studies have now shown that DDIs based on induction can be successfully studied in PCIS. Induction of expression and activity of DMEs by β-naphthoflavone, dexamethasone, rifampicin, phenobarbital, indirubin, budesonide, pregnenolone-16a carbonitrile, and 1,25$(OH)_2D_3$ were shown in PCIS of rat and human tissue.[27,51,57] These substrates are ligands for the most relevant NRs such as the aryl hydrocarbon receptor (AhR), the glucocorticoid receptor (GR), the pregnane X receptor (PXR), the vitamin D receptor (VDR), and the constitutive androstane receptor (CAR). The regulation appeared different for the different segments of rat and human intestine, and the changes did not parallel the expression levels of the NR.[57] The results of these studies showed that PCIS can be used for predictions of DDIs that are mediated by most of the relevant NRs such as AhR, GR, PXR, VDR, and CAR.

Khan et al. studied the regulation of mRNA expression of bile acid transporters, for example, organic solute transporter α/β (OSTα/β), MRP2, MRP3, and ASBT in rat jejunum, ileum and colon and in human ileum.[56] The results showed that the mRNA expression of OSTα/β was positively regulated by FXR and GR ligands in rat and human tissue but negatively regulated by 1,25$(OH)_2D_3$, a VDR ligand, in rat intestine but not in human ileum.[56] The FXR/VDR ligand lithocholic acid (LCA) was found not only to regulate the expression of these bile acid-related transporters in a species- and organ-dependent manner, but also influence the expression of the enzymes related to bile acid metabolism (CYP3A isoforms), synthesis (CYP7A1), and related NRs,[59] showing profound species differences and a complex network of induction.[57]

As discussed above, PCIS have greatly contributed to extend the knowledge about the involvement of NRs in the regulation of DTs and DMEs. The potential of rat and human PCIS as a model to detect inhibition-based DDIs has also become evident recently.[10,46,73,75] In conclusion, PCIS have a great potential to serve as a screening model for inducers and inhibitors of metabolism and transport .

Recently, PCIS were used to study the transport–metabolism interplay between DTs and DMEs. The most well-known interplay, between intestinal P-gp and CYP3A, has been studied in rat (manuscript in preparation) and human PCIS.[72] Selective P-gp inhibitors did not only enhance the tissue accumulation of quinidine, a dual substrate of P-gp and CYP3A, but also consequently increased the production of 3OH-quinidine. However, as many P-gp inhibitors are dual inhibitors of P-gp and CYP3A, this increase of CYP3A metabolism by P-gp inhibition is dependent on the relative inhibitory potency of the inhibitor for P-gp and CYP3A, Thus, ketoconazole inhibited CYP3A much more than P-gp and resulted in a decreased metabolism of quinidine despite increased quinidine accumulation due to P-gp inhibition. It is evident that the result of this interplay is strongly dependent on the relative expression of P-gp and CYP3A, which is different in the different intestinal regions. To fully predict and understand the *in vivo* effects of this interplay, PCIS obtained from all different regions should be applied. Studies with Caco-2 cells or organoids may be less useful here as they do not express these different ratios of P-gp and CYP3A .

3.4. Application of PCIS on drug toxicity

Drug-induced organ injury is a significant safety issue in pharmaceutical development and clinical therapy.[78] As a multifaceted process, it involves numerous cell types and mediators, and is often mediated by formation of reactive metabolites, and thus cannot be well-studied in *in vitro* cell cultures, but requires models that represent the multicellular, structural, and functional features of *in vivo* tissue. Moreover, such models should be viable for at least 24 h to allow toxicity to develop. Therefore, PCTS, which meet these requirements, are increasingly investigated as an alternative and promising model for elucidating the mechanisms of drug-induced organ injury.

Key target organs for drug-induced toxicity often are those highly exposed to the toxin and include the liver, kidney, and intestine. As drug-induced liver injury (DILI) is one of the main reasons for failures in clinical trials and the withdrawal of drugs from the market, toxicity on liver/hepatocytes has been widely studied. Meanwhile, drug-induced gut injury (DIGI) remains largely unstudied during preclinical investigations. This is partly because the syndromes of DIGI, for example, abdomen discomfort, ulcer, diarrhea, and bleeding, are relatively mild compared to DILI. Another reason may be that the intestinal epithelium is the most rapidly self-renewing tissue in adult mammals due to the continuous proliferation and differentiation of stem cells in the crypts. As a result, the mild-to-medium-injured intestine can be rapidly repaired by the regeneration of enterocytes after several days of injury.

Although the syndromes of DIGI are mild, its prevalence is actually high and causes considerable discomfort to patients. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) can cause a high rate (55–75%) of abnormalities on the lower GIT,[79,80] whereas bleeding and ulceration occur in the upper GIT.[81] However, it is surprising that the intestinal toxicity of drugs is so little investigated preclinically, which is at least partly due to the lack of suitable *in vitro* models. Most of the existing models do not have a sufficient viability and/or lack the appropriate DME and DT expression. Therefore, PCIS were recently presented as a model to study DIGI.[68,73,75] DIGI by NSAIDs [73,75,82] and paracetamol [82] was evaluated with rat and human PCIS using ATP depletion and morphological evaluation of changes together with the biomarkers of ER stress, mitochondrial injury, and oxidative stress as indicators, and their relative toxic potential was shown to be largely in line with the *in vivo* toxicity [73] (Figure 3). The injury to the epithelial cells by diclofenac was shown to occur at lower concentrations in rat than in human PCIS. Interestingly, it could be shown that the diclofenac metabolites did not contribute substantially to the gut injury,[73,75] but that diclofenac is directly toxic to the intestine.

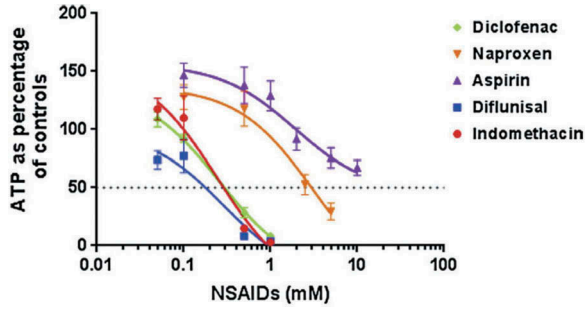

Figure 3. Nonsteroidal anti-inflammatory drug (NSAID)-induced toxicity in precision-cut intestinal slices (PCIS). NSAIDs (diflunisal, indomethacin, diclofenac, naproxen, and aspirin) induce concentration-dependent decrease of ATP in rat PCIS after 5 h of incubation. Data are normalized to the vehicle controls (5 h incubation without compounds). Data represent the average ± SEM (n ≥ 5). Reproduced with permission from [73].

Furthermore, DIGI by ketoconazole was observed in rat and human PCIS (unpublished data) in line with the clinical reports about the gut side effects of ketoconazole. In addition, PCTS from rat colon, liver, and kidney were used to test the toxicity of new potent anticancer gold(I) compounds on healthy organs. Monitored by the depletion of intracellular ATP, the colon was shown to be more resistant to the two metallocompounds than the liver and kidney.[44]

Also, several bile acids are well known for their intestinal cytotoxicity, such as LCA and DCA.[83] While the nontoxic bile acids, taurocholic acid and cholic acid, had no influence on the intracellular ATP and morphology of rat PCIS after 1 h incubation with concentrations up to 2 mM, rapid loss of ATP and morphological change was induced by DCA within 10 min,[69] as shown in Figure 4. Future research is needed to further elucidate the potential of the PCIS to predict toxicity of drugs and other xenobiotics in the human intestine and to reveal the mechanisms of DIGI.

3.5. Other applications of PCIS

A promising new application of PCTS is in studying interorgan interactions, such as intestinal induction and/or toxicity of hepatic metabolites, or vice versa, by co-incubation of PCTS from different organs. This was investigated for the first time with a microfluidic device, perfusing an intestinal slice and a liver slice sequentially (Figure 5), showing that chenodeoxycholic acid could upregulate the expression of fibroblast

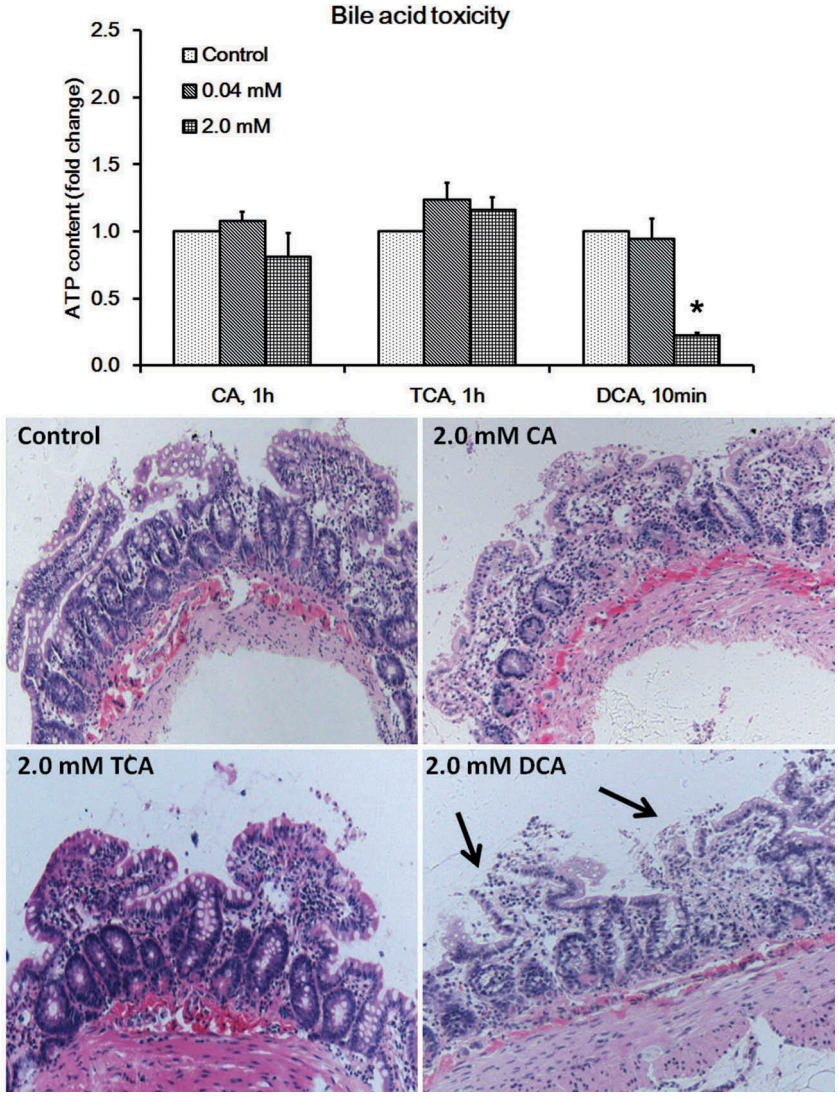

Figure 4. The toxicity of bile acids cholic acid (CA), taurocholic acid (TCA) for 1 h and deoxycholic acid (DCA) for 10 min in rat precision-cut intestinal slices. Upper panel: a significant decrease of ATP was seen already after incubation for 10 min with 2.0 mM DCA, whereas 2.0 mM CA and TCA were not toxic even after 1 h DCA. Lower panel: DCA, but not CA and TCA, caused flattening of epithelial cells and discontinuous epithelial lining (haematoxylin and eosin staining).

growth factor 15 by the intestinal slice, which resulted in a downregulation of the bile acid synthesis enzyme, CYP7A1, in the liver slices.[48]

New applications of PCIS as disease model have also been explored. Intestinal diseases, such as Crohn's disease, colitis, intestinal fibrosis, and inflammatory bowel disease, are a major health burden. However, the mechanisms of many diseases are still not fully understood, partly due to the lack of relevant *in vitro* and *in vivo* models. Recently, Pham et al. showed the application of PCIS of rat, mouse, and human intestine as a novel *ex vivo* model that can mimic the early onset of the fibrosis process in the intestine [40] by monitoring several fibrosis marker genes. TGF-β1 was found to induce the gene expression of the fibrosis markers in rat and mouse PCIS but not in human PCIS. Furthermore, PCIS were recently prepared from the fibrotic intestine of Crohn's disease patients to study late-stage human intestinal fibrosis and to test the efficacy of antifibrotic drugs.[84]

PCIS from chicken embryo intestine were used to study the infection by avian influenza virus.[28] The intestinal epithelial cells in chicken embryo PCIS remain viable for up to 4 days and are thus suitable for such infection studies. The epithelial cells at the tips of the villi were shown to be susceptible to infection by an avian influenza virus of the H9N2 subtype.

Finally, it could be very interesting to investigate the potential of PCIS to study the microbiome–tissue interactions, but no such studies have been published to date. But similar co-culture studies have been performed in other models using cell lines from intestine.[85]

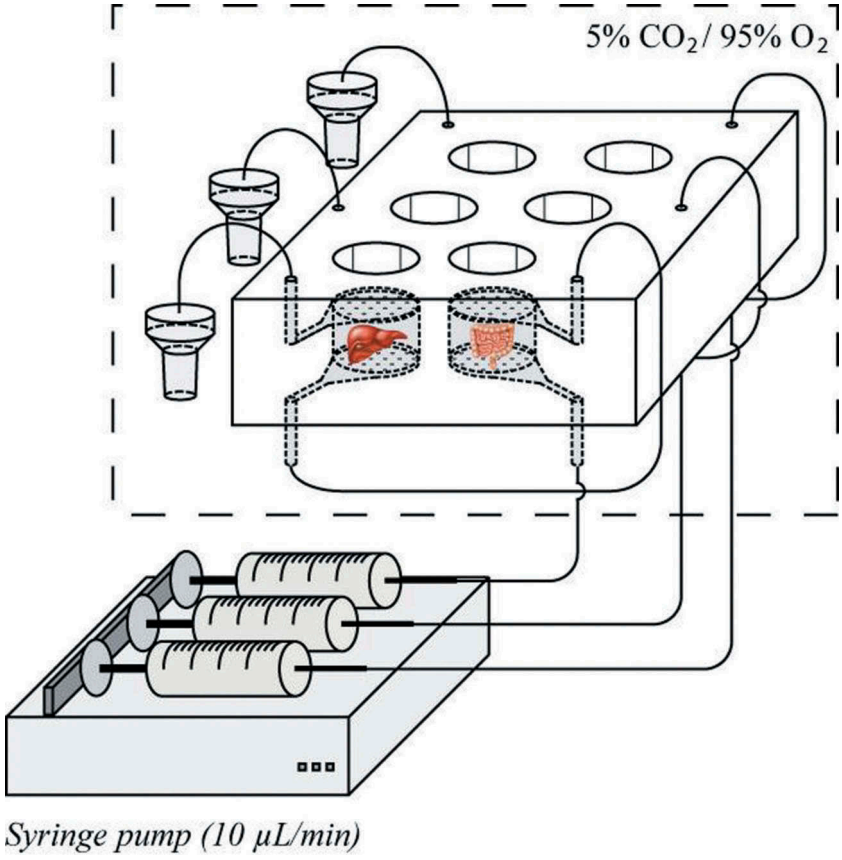

Figure 5. Schematic view of the biochip with three sets of two sequentially perfused chambers, with medium flowing (flow rate of 10 µL/min) first through the chamber with an intestinal slice and subsequently through the chamber with a liver slice. Modified from [48].

4. Limitations

Like all *in vitro* and *ex vivo* models, the PCIS model has certain limitations.

Due to the perpendicular cutting of the PCIS, both the mucosal and serosal side of the tissue are exposed to the same medium. As a result, vectorial transport of drugs across the gut wall cannot be studied with PCIS. Likewise, the excretion of the parent drug and metabolite(s) to either the mucosa or serosa compartment cannot be discriminated. However, the Ussing chamber and everted sac preparations still retain this possibility. Vickers et al. recently reported the incubation of punched intestinal tissue clamped between dual-rotating chambers, in which cyclosporine A was added to the donor compartment and transported through the tissue to the acceptor compartment.[86] This technique is similar to the Ussing chamber; however, in the rotating chambers, the intestinal surface is not fully submerged in medium while the two chambers are rotating synchronously. No comparative study has been reported yet between these two systems.

Another issue that one should bear in mind is the change in expression of DTs, DMEs, and NRs during culture. These changes may influence the activities of the proteins involved in transport and metabolism in the PCIS and ultimately may affect the generation of toxic metabolites during culture. Further optimization of the culture medium might be helpful to improve the stability of the activity of the DTs and DMEs. For instance, it is known that the addition of certain bile acids (ligands of NRs) could help to maintain bile acid-related transporters and synthesis enzymes.[59] Also, the addition of indirubin, an endogenous ligand of AhR, to the incubation medium retained CYP1A1 expression during incubation.[51]

Currently, the availability of human PCIS is limited due to the scarcity of human intestinal tissue and the lack of adequate (cryo)preservation methods. This could be solved by the establishment of tissue banks together with the development of good (cryo)preservation protocols for PCIS. The latter will also be favorable for the application of PCIS from other species like dog, monkey, or pig so that they could be optimally used at any time and at any location. The relatively short time that intestinal tissue can be preserved without variable degrees of tissue injury is also a big

challenge for small bowel transplantation, mainly due to the sensitive nature of the intestine to the ischemia-reperfusion injury.[87] The research with PCIS would greatly benefit from both improved cold preservation methods and a suitable cryopreservation protocol. Till now, no cryopreservation technique has been reported that can assure good viability and activity of PCIS after storage.

The relative short maintenance of viability of PCIS up to 24 h limits its application in chronic toxicity testing. Better culture conditions should be developed to improve the lifetime of the slices. New promising models have recently been developed such as the 'gut-on-a-chip' and intestinal organoids with longer survival time, but up to now they have not been fully characterized with respect to ADME-tox properties. Sato et al. developed an interesting *in vitro* intestinal model in which stem cells can differentiate into enterocytes, goblet cells, and enteroendocrine cells under the proper conditions and furthermore self-organize into organoids with a crypt-like domain and villus structures with a long-term survival of several months.[39] These intestinal organoids may serve as a physiologically relevant alternative method for large- and mid-scale *in vitro* testing of intestinal epithelium-damaging drugs and toxins and for the investigation of cell death pathways.[88] However, the feasibility of its applications to ADME needs further exploration and validation as the expression and activity of DMEs and DTs in these intestinal organoids are unknown, but can at best represent one of the regions of the intestine only.

5. Translational importance

As described in the sections above, many differences in drug transport, metabolism, and toxicity in the intestine have been identified between experimental animals and human, making predictions from animal studies to the human situation hazardous. The data collected so far from studies with human PCIS clearly indicate that this *ex vivo* model has the potential to predict these functions for the human intestine both for drugs and for other xenobiotics such as food compounds and environmental contaminants. Because the PCIS can be made from each of the regions of the human intestine, a better translation to the *in vivo* situation can be made compared to the use of human cell lines. In addition, mechanistic studies on drug- and xenobiotic-induced intestinal toxicity can be performed in human tissue, which provide useful data for the interpretation of clinical findings and for the risk/benefit assessment of new drugs and food components and the risk assessment for environmental toxins.

6. Conclusions

PCIS are a simple, fast, and reliable *ex vivo* model to study the transport, metabolism, and toxicity of drugs taking advantage of the physiological expression of intestinal transporters and metabolizing enzymes. This model can also be used to predict transporter- and enzyme-mediated DDIs. Moreover, it is notably valuable to study the regional gradients of activity of intestinal transporters and metabolizing enzymes from the duodenum to the colon. Furthermore, the same technique can be applied to the intestine from human and experimental animals. Therefore, it is also a very useful tool to investigate species differences in ADME-tox profiles.

7. Expert opinion

PCIS are discussed and highlighted as a promising method for drug ADME-tox studies in the intestine to be used as alternative to the *in vitro* cell culturing and *in vivo* animal modeling, which are conventionally used in academia and industry. They show relatively good viability and functionality up to 8–24 h of incubation.

Due to the physiological levels of DTs and DMEs in PCIS, at least during the first hours of culturing, they can provide physiologically relevant results, including metabolic profiles, induction and inhibition potency, and local drug/metabolite exposure, and can represent the functional differences between duodenum, jejunum, ileum, and colon. Therefore, PCIS are expected to serve as a translational model and a bridge between animal and human. The development of substrates and inhibitors with higher specificity for the enzymes and transporters could be instrumental to identify the influence of the proteins involved in the disposition of a certain substrate. In addition, PCIS made from the intestine of genetic-deficient animals such as the $Mrp2^{-/-}$ rat or genetically modified animals (i.e. Mdr1a knockout mouse) can be helpful to identify transporters involved in the transport of a drug under study.[74]

The sensitive nature of the intestine necessitates to monitor the quality of the intestine and the viability of PCIS using viability markers, for example, the intracellular ATP and morphology, in each experiment and for every compound of interest. Moreover, a further optimization of culture medium would be very useful for maintenance not only of viability but also of DME and DT activity. Addition of supplementary compounds, such as ligands for the nuclear factors involved in regulation of the DME and DT, into the medium to

optimize the culture conditions of PCIS should be investigated for each species separately since rat PCIS seem more vulnerable in the currently used medium (William's medium E) compared to mouse and human PCIS.

Extension of the viability is also instrumental for studies on DIGI, which were up to now limited to the short-term effects of NSAIDs and bile acids. Future applications may lead to the discovery of mechanisms of damage using transcriptomics technologies and of new protecting drugs for ischemia-reperfusion injury, which would be useful also for transplantation purposes.[53]

The very efficient use of human tissue makes the PCIS the preferred model for translational studies. However, as human intestinal tissue is scarce and not widely available, PCIS may be also applied to identify other animal species that in some aspects may be more similar to human than rat or mouse, for instance, monkey or (mini)pig.

The wider application of PCIS would certainly benefit from the development of better cold- and cryopreservation techniques. Development of long-term preservation technologies for PCIS hopefully will benefit from the fast-growing knowledge of cryopreservation techniques. When cryopreserved viable PCIS, especially human PCIS, become available at any time and location, a larger application in academia and industry for the ADME-tox tests during drug development will emerge.

The co-culture of PCIS with intestinal bacteria will be a promising new area since the microbiome plays an important role in the intestinal metabolism and toxicity. The gut flora can contribute to the generation of toxic metabolites that may injure the intestine.[89] The gut microenvironment was also incorporated in the 'human gut-on-a-chip' microdevice [90,91] in which Caco-2 cells formed four different types of differentiated epithelial cells and recapitulated the structure of intestinal villi. In this system, the gut microenvironment was recreated with flow of fluid and peristaltic motions, together with the co-culture of normal intestinal bacteria on the luminal surface.

Selection of slices with and without Peyer's patches, aggregations of lymphoid cells that are usually found in the ileum, may allow us to investigate the influence of the immunological function of the Peyer's patches on the intestinal metabolism and transport function and on DIGI.

Declaration of interest

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